

**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

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| **Code assigned:** | **2021.010F** |  |
| **Short title:** Create two new orders, three new families and 21 new genera in the class *Arfiviricetes* (*Cressdnaviricota*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| Fungal and Protist Viruses Subcommittee Chair |

**ICTV study group comments and response of proposer**

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**Authority to use the name of a living person**

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| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

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| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

|  |  |
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| Date first submitted to SC Chair | 05-28-2021 |
| Date of this revision (if different to above) | 10-09-2021 |

**ICTV-EC comments and response of the proposer**

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| **EC Comment:** Please address problem with Excel file (line 46)  **Response:** Done. |

**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2021.010F.A.v1.Cressdna\_2neword\_3newfam\_21newgen.xlsx |

**Abstract**

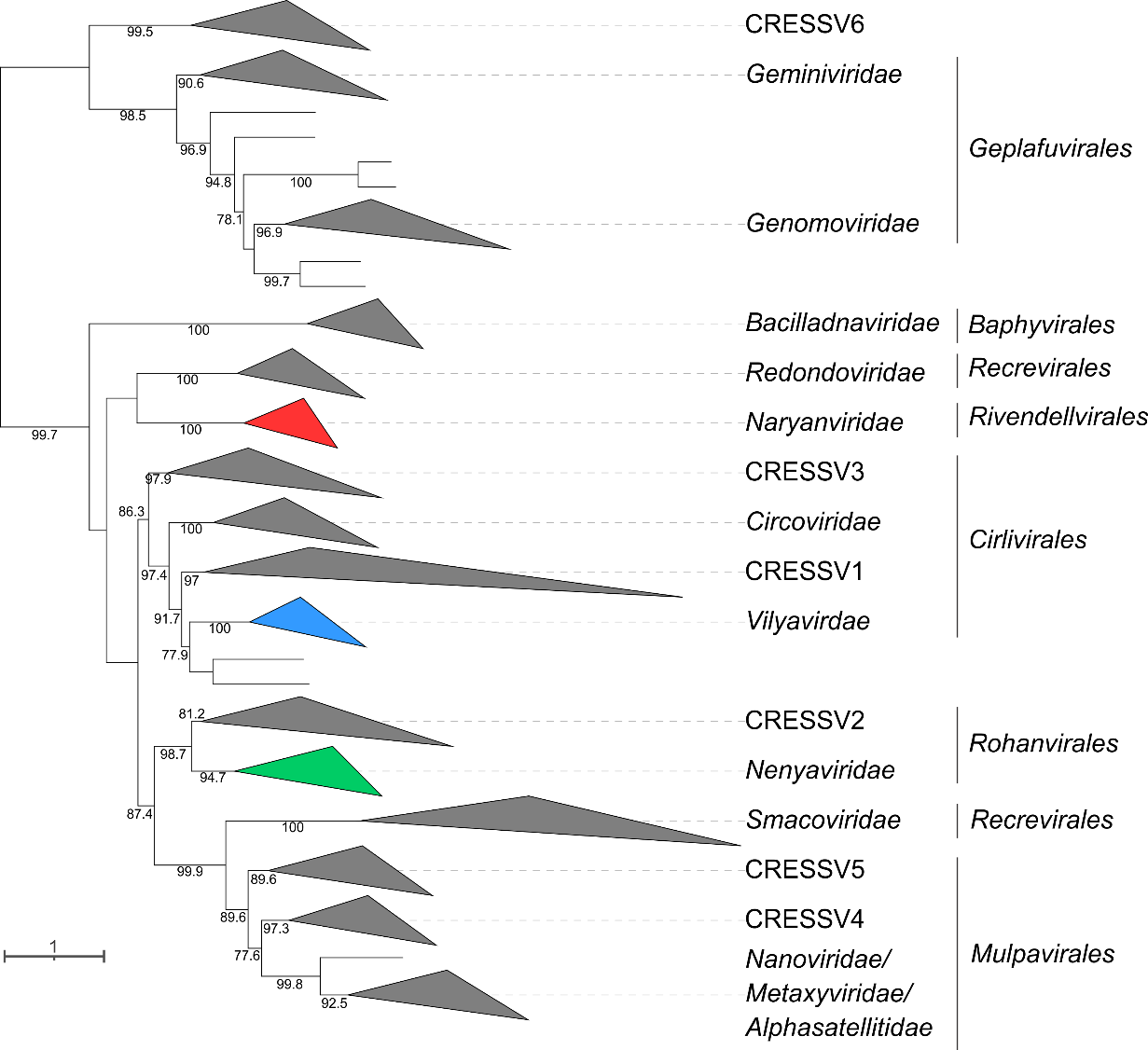
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| Here we propose creating 2 new orders (*Rivendellvirales* and *Rohanvirales*), 3 new families (*Naryaviridae*, *Nenyaviridae* and *Vilyaviridae*), with 21 new genera in the class *Arfiviricetes* (phylum *Cressdnaviricota*) for classification of single-stranded DNA viruses associated with protozoan parasites of the genera *Entamoeba* and *Giardia*. |

**Text of proposal**

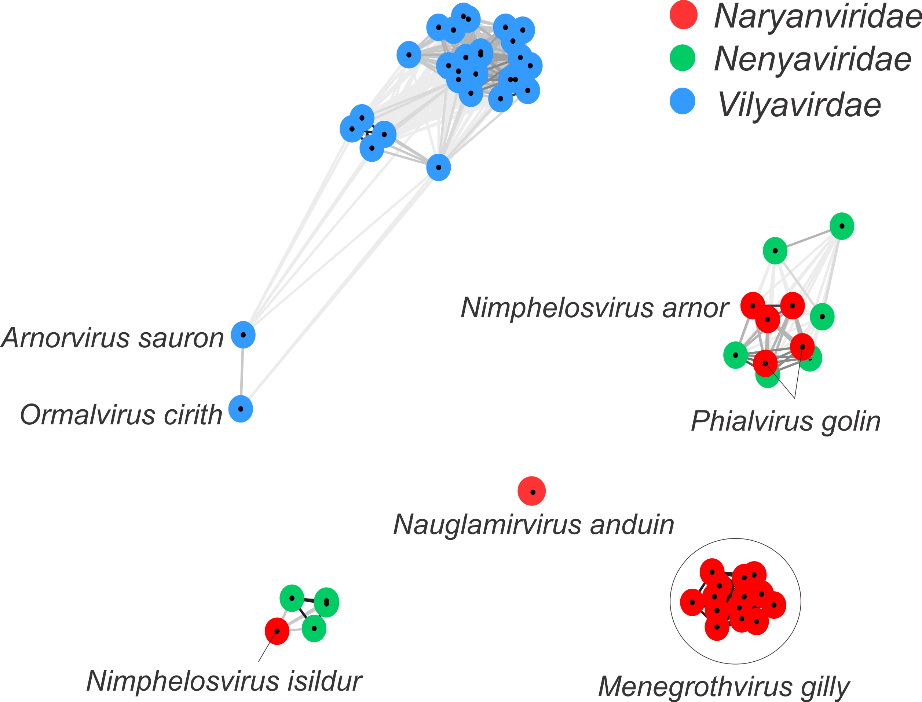
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| --- | --- |
| |  | | --- | | Phylum *Cressdnaviricota*, created in 2019, includes viruses with single-stranded DNA (ssDNA) genomes and icosahedral capsids, which infect diverse eukaryotes, including algae, fungi, plants, insects and vertebrates [6]. A characteristic feature of viruses in this phylum is the presence of homologous Rep proteins with an N-terminal rolling-circle replication initiation endonuclease domain of the HUH superfamily and a C-terminal superfamily 3 helicase domain. By contrast, the capsid proteins encoded by viruses from different families can be non-orthologous. The phylum consists of two classes, class *Repensiviricetes*, which currently includes all fungal and plant viruses of the families *Genomoviridae* and *Geminiviridae*, and class *Arfiviricetes*, which includes six virus families (*Bacilladnaviridae*, *Circoviridae*, *Smacoviridae*, *Nanoviridae*, *Metaxyviridae* and *Redondoviridae*). However, many groups of related ssDNA viruses, informally referred to as CRESSV1 to CRESSV6, remain officially unclassified [3, 4]. Recently, three groups of ssDNA viruses associated with protozoan parasites of the genera *Entamoeba* and *Giardia* have been described [5]. It has been suggested that viruses associated with the *Entamoeba* hosts could constitute two families “*Naryaviridae*” and “*Nenyaviridae*”, whereas those associated with *Giardia* hosts could form a family “*Vilyaviridae*” [5]. Here we propose to formally create and recognize the three families and to establish a sequence-based taxonomic framework and demarcation criteria for classification of these virus groups.  We collected a dataset of unclassified ssDNA virus genomes from GenBank displaying similarity to the representative members of the *Naryaviridae*, *Nenyaviridae* and *Vilyaviridae*. Maximum likelihood phylogenetic analysis of the corresponding Rep proteins in the framework of other members of the phylum *Cressdnaviricota* confirmed that the three groups form monophyletic clades distinct from the previously classified viruses (Figure 1). Viruses of the *Vilyaviridae* encode capsid proteins resembling those characteristic of circovirids, consistent with their placement within the *Cirlivirales*. By contrast, members of *Naryaviridae* and *Nenyaviridae* formed several mixed clusters of non-orthologous proteins in the network analysis (Figure 2), indicating recombination between the corresponding virus genomes and replacement of the capsid gene on several occasions, consistent with the phylogenetic analysis reported previously [5].  To analyze the relationships between viruses within each of the three groups, we focused on all-against-all genome and Rep comparisons as well as phylogenetic analysis (Figures 3-5). For genus demarcation, we used 65% pairwise nucleotide sequence identity, whereas 78% pairwise nucleotide sequence identity was chosen as a species demarcation criterion, similar to that used for other cressdnaviricots, including genomovirids [9] and smacovirids [10]. Thus, all viral genomes showing identities higher than 78% should be considered as variants of the existing species. Nonetheless, there may be situations where it is difficult to assign certain viruses to clearcut species because a particular new sequence is  1. >78% similar to sequences from a particular species but is <78% similar to other variants of that same species;  2. >78% similar to sequences from two or more different species.  To resolve the above conflicts, we suggest adopting a similar approach proposed for geminiviruses [1] and genomoviruses [9]. To resolve conflict 1, we suggest that the new virus be classified within any species in which it shares >78% sequence identity to any one variant formerly classified as belonging to that same species, even if it is <78% identical to other viruses within that species. To resolve conflict 2, we suggest that the new sequence be considered as belonging to the species with sequences with which it shares the highest degree of similarity.  Using these criteria, *Vilyaviridae* was divided into 12 genera with 18 species (Figure 3); *Naryaviridae* was divided into 4 genera with 5 species (Figure 4), whereas *Nenyaviridae* includes 5 genera with 6 species (Figure 5). Maximum likelihood phylogenetic analysis and pairwise comparison of the Rep amino acid sequences fully recapitulate the genome identity-based classification (Figures 3-5).  Whereas *Vilyavirdae* falls within the established order *Cirlivirales, Naryaviridae* and *Nenyaviridae* form distinct branches within the *Arfiviricetes* (Figure 1). Thus, to bridge the gap between the family and class taxa, we propose creating orders *Rivendellvirales* and *Rohanvirales* which would accommodate families *Naryaviridae* and *Nenyaviridae*, respectively.  **Etymology of the taxa names**  The families *Naryaviridae*, *Nenyaviridae* and *Vilyaviridae* are named after three rings from the Middle-earth canon (also known as Tolkien’s canon) [5]. We upheld this theme when devising names of other taxa.  Orders:  ***Rivendellvirales***, after Rivendell, elven-refuge in a steep and hidden valley to the west of the Misty Mountains. Founded in the Second Age by Elrond, Rivendell was home to a number of great Elven-lords.  ***Rohanvirales***, after Rohan, kingdom of the Rohirrim, bounded by the Anduin, the Misty Mountains, and Fangorn Forest, among others; once a province of Gondor, the land was given to the Men of Eotheod in return for their aid to Gondor in a battle.  Genera:  In family ***Naryaviridae***  *Menegrothvirus*, after Menegroth, also known as the Thousand Caves, was the capital city of the land of Doriath.  *Nauglamirvirus*, after Nauglamír, a necklace given as a gift from the dwarves to Finrod Felagund of Nargothrond.  *Nimphelosvirus*, after Nimphelos, a valuable pearl which appears in The Silmarillion, given by Thingol to the Dwarves from Belegost as a reward for building Menegroth  *Phialvirus*, after Phial, a parting gift to Frodo Baggins by Galadriel, also known as the Star-glass.    In family ***Nenyaviridae***  *Angainorvirus*, after Angainor, the chain used to contain Melkor in the Halls of Mandos.  *Galvornvirus*, after Galvorn, a jet black metal devised by the Dark Elf Eöl.  *Mazarbulvirus*, after Mazarbul, which means "records" in the Dwarf-language Khuzdul, and the chamber where it was kept was similarly named.  Mithrilvirus, after Mithril, a silvery metal, stronger than steel but much lighter in weight.  *Valinorvirus*, after Valinor (Land of the Valar), a fictional location in J. R. R. Tolkien's legendarium.    In family ***Vilyaviridae***  *Andurilvirus*, after Anduril, an Elven-forged sword used to slay Sauron, Morgoth's successor.  *Angristvirus*, after Angrist, a knife made by the great weaponsmith Telchar of Nogrod.  *Aranruthvirus*, after Aranrúth, a sword wielded by King Thingol of Doriath in The Silmarillion.  *Arnorvirus*, after Arnor, the North Kingdom of the Dundain founded in the  Second Age, after the destruction of Nmenor.  *Glamdringvirus*, after Glamdring, a sword forged in the First Age by the High Elves of the hidden city of Gondolin.  Grondvirus, after Grond, the name of the mace of Morgoth in The Silmarillion; also a battering ram in The Lord of the Rings, used to assault the main gate of Minas Tirith.  *Herugrimvirus*, after Herugrim, a sword that belonged to Théoden.  *Illuinvirus*, after Illuin, one of the two great lamps which stood at the northern end of Arda.  *Ormalvirus*, after Ormal, one of the two great lamps which stood at the southern end of Arda.  *Palantirivirus*, after Palantíri, stone globes which function somewhat like crystal balls or communication devices.  *Ringilvirus*, after Ringil, a blade that has a chilling blue glow like ice reflecting the light of stars.  *Vingilotevirus*, after Vingilótë, the ship in which Eärendil and Elwing sailed to Aman to seek pardon and assistance from the Valar.  For species naming, we use binomial format with the “Genus name + free-form epithet”, where epithets are derived from various characters from the Tolkien’s canon. All species, genera and families, and their members are listed in Table 1. | |

**Supporting evidence**

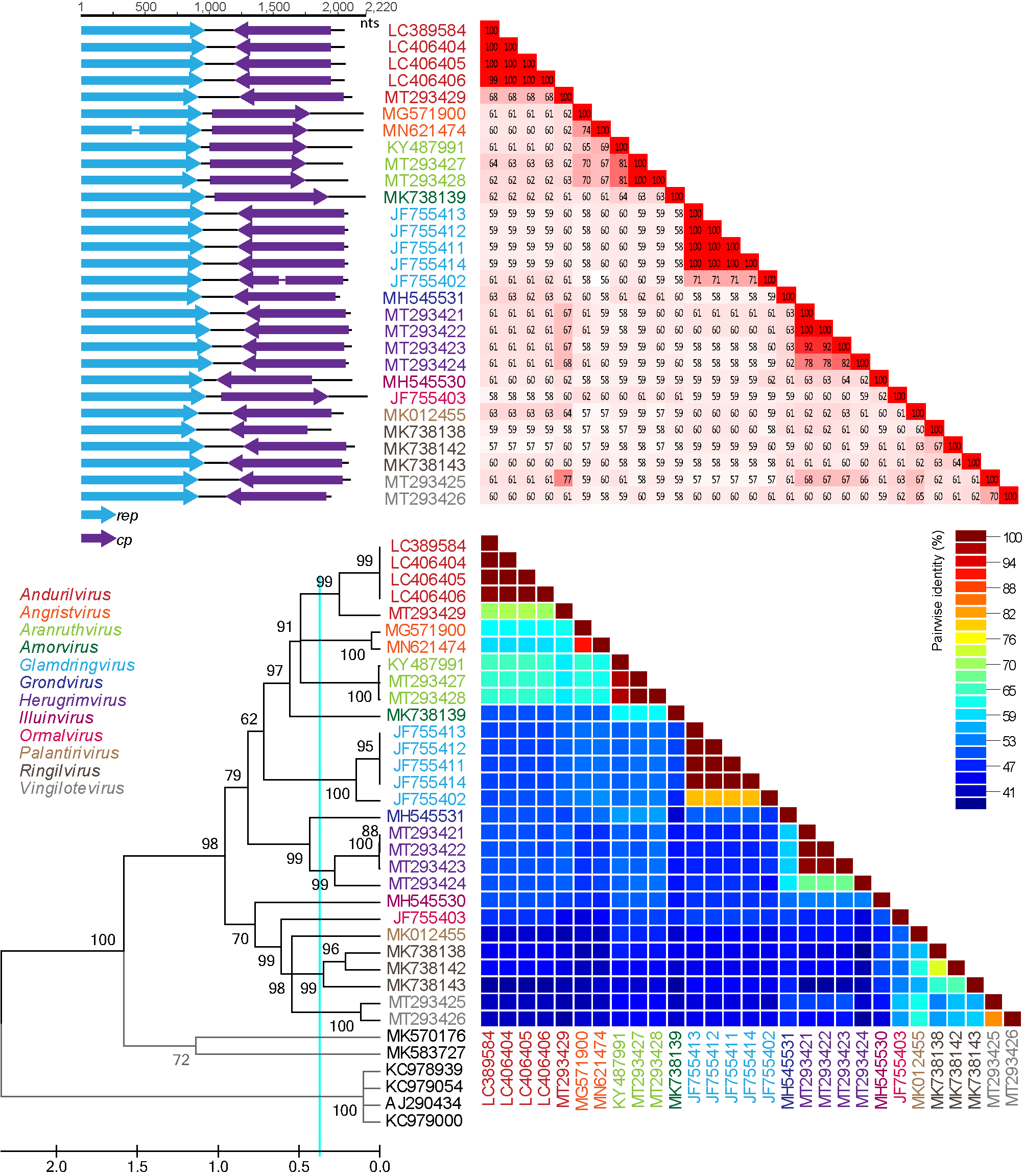
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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Family** | **Genus** | **Species** | **Accession #** | **Virus** | **Country of isolation** | **Host / source** | **Isolate** | **Sample type** |
| *Naryaviridae* | *Menegrothvirus* | *Menegrothvirus gilly* | MT181534 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR17\_3\_2236 | feces |
|  |  |  | MT181540 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR26\_3\_2236 | feces |
|  |  |  | MT181536 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR16\_3\_2236 | feces |
|  |  |  | MT181537 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR16\_1\_2236 | feces |
|  |  |  | MT181543 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR3\_1\_2236 | feces |
|  |  |  | MT181538 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR14\_3\_2236 | feces |
|  |  |  | MT181535 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR17\_1\_2236 | feces |
|  |  |  | MT181539 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR14\_1\_2236 | feces |
|  |  |  | MT181533 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR24\_1\_2236 | feces |
|  |  |  | MT181532 | Marmot associated feces virus 4 | USA | *Marmota flaviventris* | MAR24\_3\_2236 | feces |
|  |  |  | MT181531 | Marmot associated feces virus 3 | USA | *Marmota flaviventris* | MAR25\_1\_2236 | feces |
|  |  |  | MT181530 | Marmot associated feces virus 3 | USA | *Marmota flaviventris* | MAR25\_3\_2236 | feces |
|  |  |  | MT181528 | Marmot associated feces virus 3 | USA | *Marmota flaviventris* | MAR26\_3\_2236 | feces |
|  |  |  | MT181529 | Marmot associated feces virus 3 | USA | *Marmota flaviventris* | MAR26\_1\_2236 | feces |
|  | *Nauglamirvirus* | *Nauglamirvirus anduin* | MN379590 | Chicken virus mg5\_2876 | USA | *Gallus gallus* | mg5\_2876 | tracheal swab |
|  | *Nimphelosvirus* | *Nimphelosvirus isildur* | MT293413 | Entamoeba-associated CRESS DNA virus 1 | Netherlands | Entamoeba | 84-AMS-03 | human stool |
|  |  |  | MT293410 | Entamoeba-associated CRESS DNA virus 1 | Netherlands | Entamoeba | 84-AMS-01 | human stool |
|  |  |  | MT293411 | Entamoeba-associated CRESS DNA virus 1 | Netherlands | Entamoeba | 84-AMS-02 | human stool |
|  |  |  | MT293412 | Entamoeba-associated CRESS DNA virus 1 | Netherlands | Entamoeba | 94-AMS-01 | human stool |
|  | *Phialvirus* | *Phialvirus golin* | MT293414 | Entamoeba-associated CRESS DNA virus 2 | Netherlands | Entamoeba | 84-AMS-01 | human stool |
|  |  |  | MT293415 | Entamoeba-associated CRESS DNA virus 2 | Netherlands | Entamoeba | 94-AMS-01 | human stool |
| *Nenyaviridae* | *Angainorvirus* | *Angainorvirus turin* | MT293418 | Entamoeba-associated CRESS DNA virus 4 | Netherlands | Entamoeba | 84-AMS-01 | human stool |
|  |  |  | MT293420 | Entamoeba-associated CRESS DNA virus 4 | Netherlands | Entamoeba | 84-AMS-03 | human stool |
|  |  |  | MT293419 | Entamoeba-associated CRESS DNA virus 4 | Netherlands | Entamoeba | 84-AMS-02 | human stool |
|  | *Galvornvirus* | *Galvornvirus isengard* | MH617639 | Circoviridae sp. | USA | *Macaca sp* | ctga69 | macaque stool |
|  | *Mazarbulvirus* | *Mazarbulvirus hasufel* | MT293416 | Entamoeba-associated CRESS DNA virus 3 | Netherlands | Entamoeba | 84-AMS-01 | human stool |
|  |  |  | MG571899 | unidentified circular ssDNA virus | Venezuela | *Homo sapiens* | V16C | human stool |
|  |  |  | MT293417 | Entamoeba-associated CRESS DNA virus 3 | Netherlands | Entamoeba | 84-AMS-02 | human stool |
|  | *Mithrilvirus* | *Mithrilvirus smaug* | KM598397 | Odonata-associated circular virus-14 | USA | *Erythrodiplax fusca* | OdasCV-14-US-1577SC3-12 | whole insect |
|  | *Valinorvirus* | *Valinorvirus arda* | KY487839 | uncultured virus | USA | environmental | CG97 | wastewater |
|  |  | *Valinorvirus eriador* | KY487840 | uncultured virus | USA | environmental | CG171 | wastewater |
| *Vilyaviridae* | *Andurilvirus* | *Andurilvirus erebor* | LC389584 | Feline stool-associated circular virus KU14 | Japan | *Felis catus* | KU14 | feces |
|  |  |  | LC406404 | Feline stool-associated circular virus KU9 | Japan | *Felis catus* | KU9 | feces |
|  |  |  | LC406405 | Feline stool-associated circular virus KU7 | Japan | *Felis catus* | KU7 | feces |
|  |  |  | LC406406 | Feline stool-associated circular virus KU8 | Japan | *Felis catus* | KU8 | feces |
|  |  | *Andurilvirus finwe* | MT293429 | Giardia-associated CRESS DNA virus 4 | Netherlands | Giardia | 84-AMS-01 | human stool |
|  | *Angristvirus* | *Angristvirus hurin* | MG571900 | unidentified circular ssDNA virus | Venezuela | *Homo sapiens* | V18B | human stool |
|  |  | *Angristvirus fangorn* | MN621474 | CRESS virus sp. | China | *Moschus berezovskii* | UJSL012 | tissue |
|  | *Aranruthvirus* | *Aranruthvirus numenor* | KY487991 | uncultured virus | USA | environmental | CG97 | wastewater |
|  |  |  | MT293427 | Giardia-associated CRESS DNA virus 3 | Netherlands | Giardia | 84-AMS-01 | human stool |
|  |  |  | MT293428 | Giardia-associated CRESS DNA virus 3 | Netherlands | Giardia | 84-AMS-02 | human stool |
|  | *Arnorvirus* | *Arnorvirus sauron* | MK738139 | Northern red-backed vole stool-associated circular virus 16 | Russia | *Myodes rutilus* | MR-16 | feces |
|  | *Glamdringvirus* | *Glamdringvirus thorin* | JF755413 | Rodent stool-associated circular genome virus | USA | *Microtus pennsylvanicus* | RodSCV\_V-84 | feces |
|  |  |  | JF755412 | Rodent stool-associated circular genome virus | USA | *Microtus pennsylvanicus* | RodSCV\_V-81 | feces |
|  |  |  | JF755411 | Rodent stool-associated circular genome virus | USA | *Microtus pennsylvanicus* | RodSCV\_V-72 | feces |
|  |  |  | JF755414 | Rodent stool-associated circular genome virus | USA | *Microtus pennsylvanicus* | RodSCV\_V-97 | feces |
|  |  | *Glamdringvirus minas* | JF755402 | Rodent stool-associated circular genome virus | USA | *Microtus pennsylvanicus* | RodSCV\_M-89 | feces |
|  | *Grondvirus* | *Grondvirus lorian* | MH545531 | Fly associated circular virus 7 | Saint Barthelemy | Blow flies (Lucilia sp) | Barts\_I1021\_F10 | Whole insect |
|  | *Herugrimvirus* | *Herugrimvirus gladden* | MT293421 | Giardia-associated CRESS DNA virus 1 | Netherlands | Giardia | 84-AMS-01 | human stool |
|  |  |  | MT293422 | Giardia-associated CRESS DNA virus 1 | Netherlands | Giardia | 84-AMS-02 | human stool |
|  |  |  | MT293423 | Giardia-associated CRESS DNA virus 1 | Netherlands | Giardia | 84-AMS-03 | human stool |
|  |  |  | MT293424 | Giardia-associated CRESS DNA virus 1 | Netherlands | Giardia | 84-AMS-04 | human stool |
|  | *Illuinvirus* | *Illuinvirus amon* | MH545530 | Fly associated circular virus 6 | Guadeloupe | Blow flies (Lucilia sp) | GP\_I1020-I75\_F12 | Whole insect |
|  | *Ormalvirus* | *Ormalvirus cirith* | JF755403 | Rodent stool-associated circular genome virus | USA | Microtus pennsylvanicus | RodSCV\_V-69 | feces |
|  | *Palantirivirus* | *Palantirivirus imlardis* | MK012455 | CRESS virus sp. | USA | Sheep | ctjj384 | lamb tissue |
|  | *Ringilvirus* | *Ringilvirus tuor* | MK738138 | Northern red-backed vole stool-associated circular virus 11 | Russia | *Myodes rutilus* | MR-11 | feces |
|  |  | *Ringilvirus thingol* | MK738142 | Northern red-backed vole stool-associated circular virus 116 | Russia | *Myodes rutilus* | MR-116 | feces |
|  |  | *Ringilvirus ungol* | MK738143 | Northern red-backed vole stool-associated circular virus 117 | Russia | *Myodes rutilus* | MR-117 | feces |
|  | *Vingilotevirus* | *Vingilotevirus gimli* | MT293425 | Giardia-associated CRESS DNA virus 2 | Netherlands | Giardia | 84-AMS-01 | human stool |
|  |  | *Vingilotevirus gamgee* | MT293426 | Giardia-associated CRESS DNA virus 2 | Netherlands | Giardia | 84-AMS-02 | human stool |

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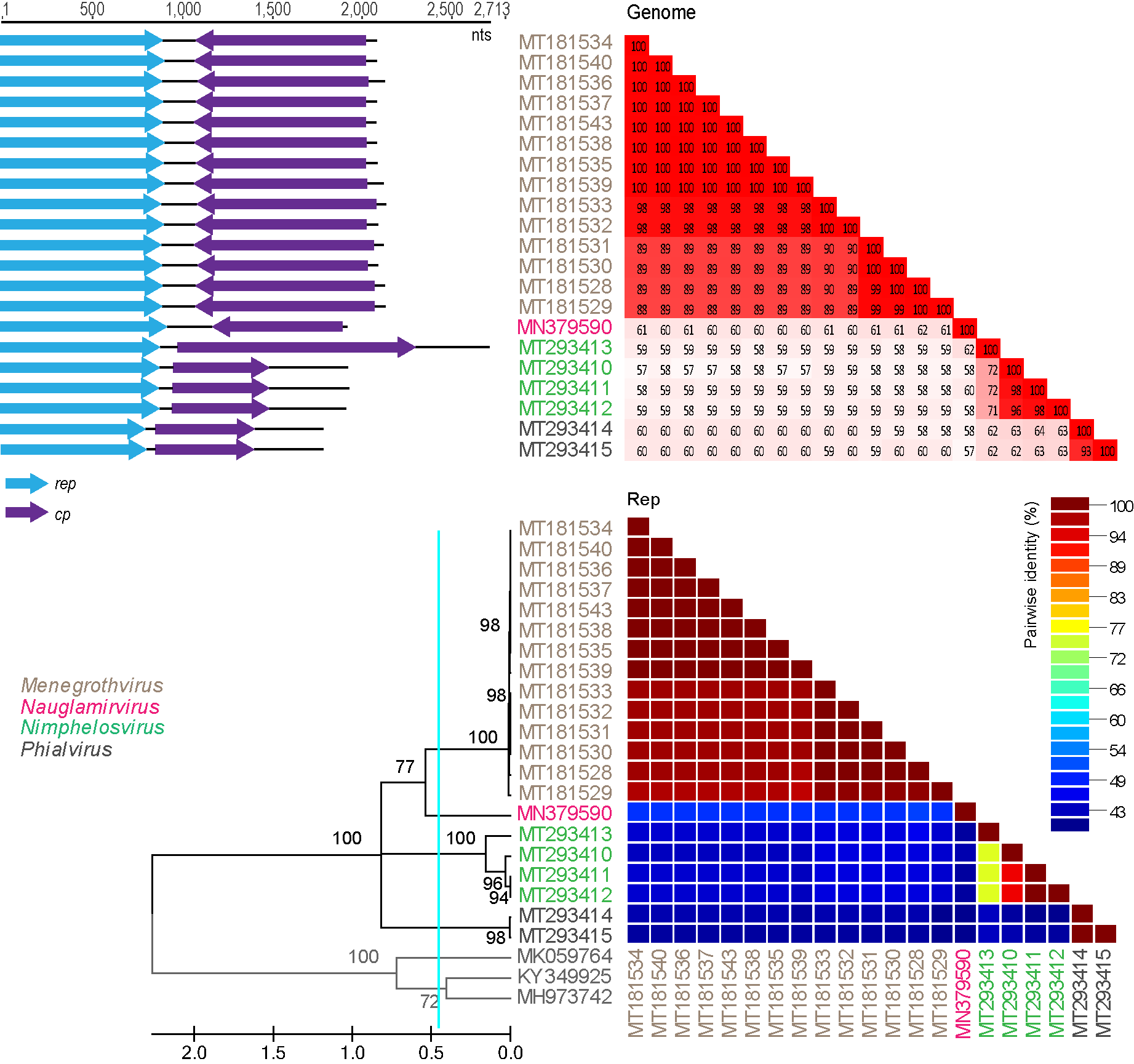
**Figure 1.** Maximum likelihood phylogenetic tree of Rep proteins from members of the phylum *Cressdnaviricota*. Closely related sequence groups are collapsed into triangles, the side lengths of which are proportional to the distances between the closest and farthest leaf nodes. The Rep alignment used for the tree reconstruction was taken from ref [6] and supplemented with sequences of members of the proposed families *Naryaviridae*, *Nenyaviridae* and *Vilyaviridae*. The latter dataset included previously reported sequences [5] as well as related sequences downloaded from GenBank. The alignment was trimmed with TrimAL [2] with gap threshold of 0.2. The maximum likelihood phylogenetic tree was constructed using IQtree [7] with automatic selection of the best-fit substitution model for a given alignment, which was rtREV+F+R6. Numbers at the nodes represent aLRT branch supports. The scale bar represents the number of substitutions per site.

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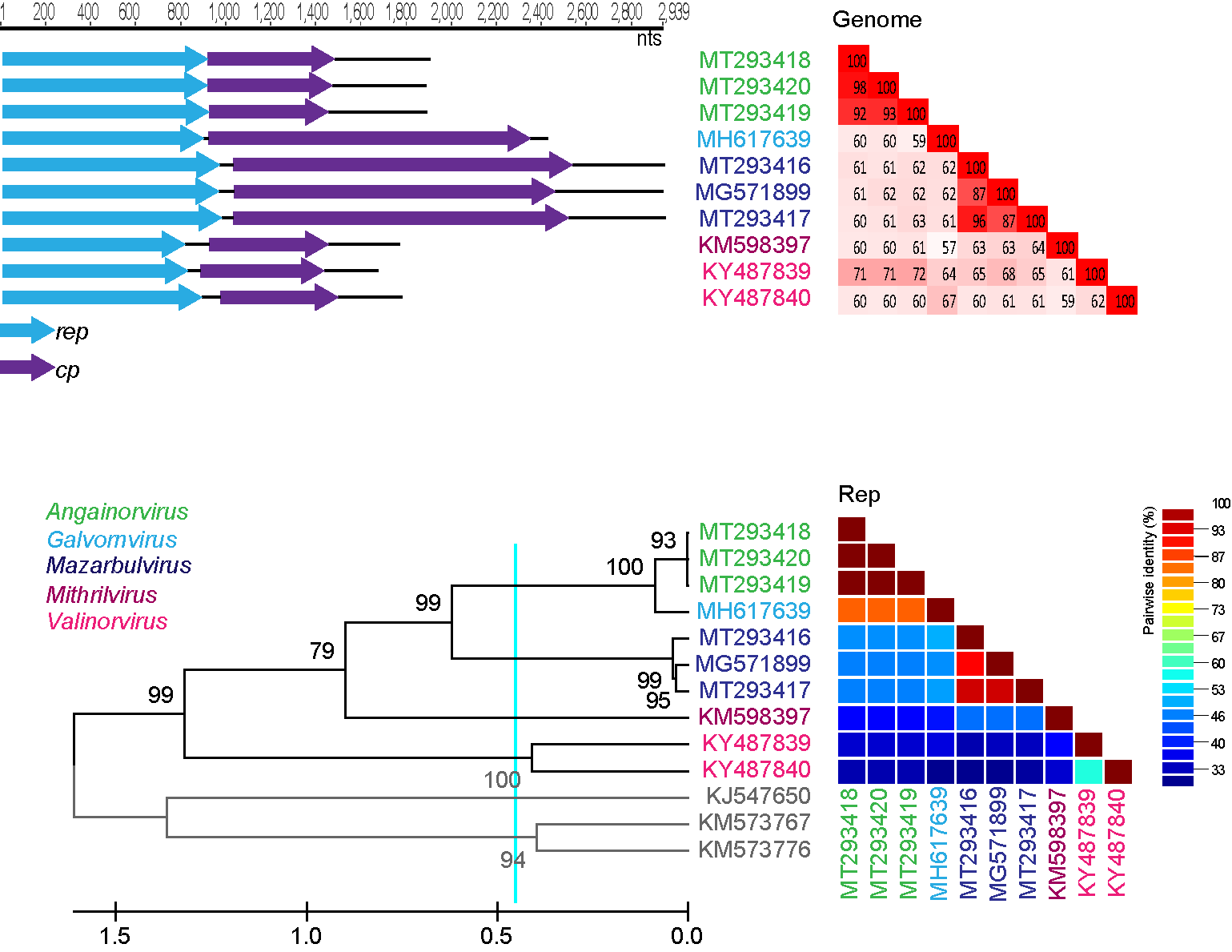
**Figure 2.** Diversity of capsid proteins encoded by members of the families *Naryaviridae,* *Nenyaviridae* and *Vilyaviridae*. Protein sequences were clustered by their pairwise sequence similarity. Lines connect sequences with P-value ≤ 1e−05. Species of the *Naryaviridae*, which form several distinct clusters are labeled.



**Figure 3.** Genera and species of the family *Vilyaviridae*. Top: Pairwise all-against-all comparison of the vilyavirid genomes. Genome maps are shown on the left, whereas a heatmap showing the corresponding identity values is shown on the right. Bottom: Maximum likelihood tree of the *Vilyaviridae* (left) and heatmap showing pairwise comparison of the Rep amino-acid sequences (right). Species belonging to the same genus are indicated with the same color; the genera are listed on the right. The phylogeny was inferred using IQtree [7], with automatic selection of the best-fit substitution model for a given alignment, which was Q.pfam+F+I+G4. Numbers at the nodes represent aLRT branch supports. The cyan line shows a proposed demarcation of genera. The pairwise identities were calculated using SDT [8].

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**Figure 4.** Genera and species of the family *Naryaviridae*. Top: Pairwise all-against-all comparison of the vilyavirid genomes. Genome maps are shown on the left, whereas a heatmap showing the corresponding identity values is shown on the right. Bottom: Maximum likelihood tree of the *Naryaviridae* (left) and heatmap showing pairwise comparison of the Rep amino-acid sequences (right). Species belonging to the same genus are indicated with the same color; the genera are listed on the right. The phylogeny was inferred using IQtree [7], with automatic selection of the best-fit substitution model for a given alignment, which was LG+G4. Numbers at the nodes represent aLRT branch supports. The cyan line shows a proposed demarcation of genera. The pairwise identities were calculated using SDT [8].

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**Figure 5.** Genera and species of the family *Nenyaviridae.* Top: Pairwise all-against-all comparison of the vilyavirid genomes. Genome maps are shown on the left, whereas a heatmap showing the corresponding identity values is shown on the right. Bottom: Maximum likelihood tree of the *Nenyaviridae* (left) and heatmap showing pairwise comparison of the Rep amino-acid sequences (right). Species belonging to the same genus are indicated with the same color; the genera are listed on the right. The phylogeny was inferred using IQtree [7], with automatic selection of the best-fit substitution model for a given alignment, which was LG+G4. Numbers at the nodes represent aLRT branch supports. The cyan line shows a proposed demarcation of genera. The pairwise identities were calculated using SDT [8].

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